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Floral biology and pollination in *Diospyros chloroxylon* (Ebenaceae) and *Hybanthus enneaspermus* (Violaceae)

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ABSTRACT

Diospyros chloroxylon is an evergreen dry season blooming tree species. It is dioecious with male and female flowers, each possessing non-functional opposite sex organ, which is an indication that this sexual system is evolved from hermaphroditism. Male flowers provide both nectar and pollen and while female flowers provide only nectar but honey bees as exclusive foragers visit both flower sexes indiscriminately effecting cross-pollination. Ornithochory is functional in this species. *Hybanthus enneaspermus* is an annual hermaphroditic wet season herb. It is self-compatible, allogamous and autogamous. Lycaenid butterflies are involved in the functionality of allogamy while flower closure event brings about autogamy. The elaborate floral mechanism controls the occurrence of autogamy to promote allogamy.

Key words:
Diospyros chloroxylon, dioecy, honey bees, nectar robbery, ornithochory, *Hybanthus enneaspermus*, allogamy, autogamy, psychophily

1. INTRODUCTION

The genus *Diospyros* has more than 700 species of deciduous and evergreen trees and shrubs, a majority of which are native to tropical latitudes while a few species are also extended their distribution into temperate regions (Akagi et al. 2013). The genus name is derived from Greek words "*dios*" and "*pyros*" meaning "Zeus's wheat" which refers to divine food or fruit (Jaeger 1959). Species in this genus are important as valuable timber sources; they are also cultivated for their ornamental and fruit value. In this genus, male flowers are smaller than female flowers. In male flowers, the stamens are many and arranged in two distinguishable whorls with the inner whorl of stamens shorter than the outer one. The pistillode is represented by irregular lumps of hairy tissue without style stylodia and ovules. In female flowers, the staminodes in reduced size and shape occur in a single whorl. The ovary is syncarpous with a few biovulate carpels, extended into a short simple style or slightly bifid stylodia which are equivalent to the number of carpels (Corner 1976). This genus is reputed for its medicinal uses in traditional medicinal systems (Mallavadhani et al. 1998; Bhushan et al.

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2005). Since *Diospyros* has commercial, ornamental and edible values, different workers investigated different aspects of reproductive ecology of some species in this genus to understand the dependence of these species on pollinator fauna due to high prevalence of dioecious sexual system. *D. virginiana* is visited by bees (Hague 1911), *D. lyciodes* by bees, wasps and butterflies, *D. dichrophylla* by sunbirds (Pooley 1993), *D. pentamera* by beetles, flies and wasps (House 1992), and *D. hispida* by nocturnal moths (Silberbauer-Gottsberger and Gottsberger 1988) and *D. blancoi* by thrips (Hung et al. 2017). In India, approximately 41 *Diospyros* species have been reported to occur (Sastry 1952). Among these species, there is sufficient knowledge on different aspects of *D. melanoxylon* due to its leaf value as it is used for making "beedis" (tobacco wrapped in the leaves). The beedis are used for smoking by people mostly hailing from lower socio-economic category (Benjamin et al. 2003). *D. chloroxylon* is most diverse in the rainforests of Tropical Africa, Tropical America, Malaysia and India. It is widely used in traditional medicine (Sirisha et al. 2018).

The genus *Hybanthus* with about 100 species herbs, shrubs or small trees is distributed in different ecological situations throughout the tropics and also in temperate North America (Jerome and Alain 2003). In this genus, certain species such as *H. communis, H. concolor* and *H. ipecacuanha* are hermaphroditic and produce chasmogamous and cleistogamous flowers; the former type is both allogamous and autogamous while the latter type is exclusively autogamous (Brizicky 1961). In *H. prunifolius*, the flowers are hermaphroditic, self-compatible and allogamous (Beattie 1971; Kroon 1972). *H. enneaspermus* has a wide range distribution extending from west equatorial Africa to Madagascar to India, Southeast Asia and Australia (Jacobs and Moore 1971; Bennett 1972). It is also hermaphroditic and produces only chasmogamous self-compatible flowers which are adapted for the occurrence of allogamy and autogamy (Jerome and Alain 2003); it is visited only by *Ceratina* bees which effect nototribic pollination (Beattie 1974). In the present study, information on some aspects of floral biology and pollination is provided for *Diosypros chloroxylon* Roxb. (Ebenaceae) and *Hybanthus enneaspermus* (L.) F. Muell (Violaceae). The main intent of this study is to find out the extent of pollinator-dependency for pollination in these two species.

2. MATERIALS AND METHODS

Trees of *Diosypros chloroxylon* located in the dry deciduous forest ecosystem in Kadiri area in southern Eastern Ghats of Andhra Pradesh and *Hybanthus enneaspermus* growing in wild patches occurring in Adavivarm area of western Visakhapatnam city of Andhra Pradesh was selected for study during April-June 2019 and July 2020-March 2021. These two species were investigated for their flowering period, floral biology, pollination mechanism, sexual system, pollinators, and fruit and seed dispersal. These aspects were examined to understand their sexual reproduction.

3. RESULTS AND DISCUSSION

***Diospyros chloroxylon* Roxb**.: It is an evergreen tree covered with dark brown rough bark. Individual trees of this species occur scattered at the study site. It flowers during dry season from March-May but certain individuals initiate flowering in mid-February which could be attributable to early increase in local temperature due to global warming effect. It is dioecious with sessile male and female flowers in the leaf axils of different individuals. The male flowers are smaller than female flowers and borne in leaf axils. Both are white, slightly fragrant, oriented either horizontally or slightly hanging downward. The male flowers are borne in cymose clusters while female flowers are borne solitary. In both flower sexes, the calyx is campanulate with deeply divided lobes while the corolla is pitcher-shaped and apically divided into 4 lobes but rarely into 5 lobes. In male flowers, the stamens vary from 14-16 arranged in 2 rows; they are hairy and equal in size and pistillode is rudimentary. In female flowers, the stamens are replaced by 7-8 glabrous staminodes. The pistil consists of a glabrous, ovary surmounted by 4 erect glabrous styles, each with bi-fid stigma. The ovary is 4-celled and each with two ovules. Both male and female flowers are nectariferous; the former offer both nectar and pollen while the latter offer only nectar as floral reward(s) for flower-visitors. The flowers are open during early morning hours and remain so until their fall off. The honey bees, *Apis dorsata, A. cerana* (Figure 1a) and *A. florea* (Figure 1b) were the only foragers that visited the flowers throughout the day with concentrated forage collection activity confined to forenoon period. Of these, *A. dorsata* was the occasional forager while the other honey bees were regular consistent throughout the flowering period. Further, *A. cerana* and *A. florea* visited the flowers also illegitimately; in this behavior, these bees landed on the outside of corolla, moved to the flower base and pierced through the basal part of the corolla tube to access nectar (Figure 1c). These bees also exhibited this illegitimate foraging behavior on mature flower buds for nectar collection. The legitimate flower probing for forage collection by the bees was found to be transferring pollen from male to female flowers with pollination as a result. However, illegitimate flower probing representing the display of nectar robbery by these bees appeared to be promoting legitimate flower visits by them, which is bound to increase pollinate rate in female flowers.

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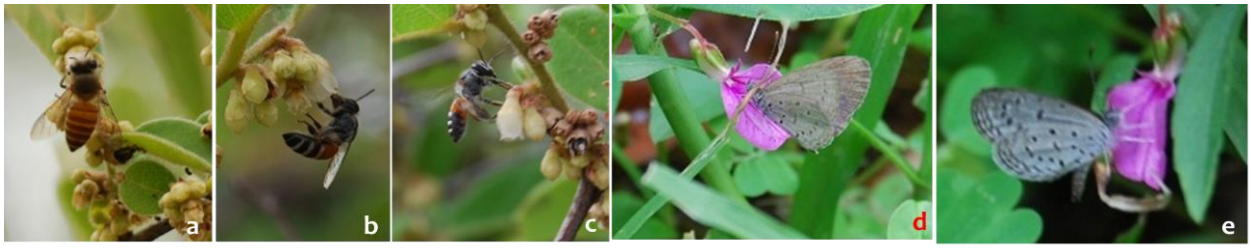

Figure 1. ***Diosypros chloroxylon:*** a. *Apis cerana*, b. *Apis florea*, c. *Apis florea* piercing the corolla base for nectar collection; ***Hybanthus enneaspermus***: Lycaenid butterflies collecting nectar – d. *Zizeeria karsandra*, e. *Zizula hylax*.

Namikawa et al. (1932) reported that *Diospyros kaki* flowers are open during early morning. Silbergauer-Gottsberger and Gottsberger (1988) reported that *D. hispida* flowers are open during night time. In this study, it is found that *D. chloroxylon* flowers are open during early morning hours as in *D. kaki* (Namikawa et al. 1932). Hague (1911) reported that *D. virginiana* is visited by bees. Pooley (1993) reported that *D. lyciodes* is visited by bees, wasps and butterflies, and *D. dichrophylla* by sunbirds. House (1992) reported that *D. pentamera* is visited by beetles, flies and wasps. Silberbauer-Gottsberger and Gottsberger (1988) reported that *D. hispida* is visited by nocturnal moths. Hung et al. (2017) reported that *D. blancoi* is visited by ants and thrips but the ants are not pollinators while the thrips represented by a single species *T. hawaiiensis* is the major pollinator. Sirisha et al. (2018) reported that *D. chloroxylon* is pollinated by a wide variety of insects but is is mainly pollinated by honey bees. In this study, *D. chloroxylon* is found to be pollinated exclusively by honey bees as they visit both male and female plants for forage collection; the pollination by only honey bees at the study site could be due to the occurrence of flowering during summer season and also non-availability of other bees and/or insects here as the study site represents deciduous scrub jungle type forest ecosystem.

White (1983) reported that in *Diosypros* genus, the seed is encircled longitudinally by a persistent, distinctly raised, straight, and sometimes branched, vascular strand. Pannell and White (1988) reported that *Diospyros* seeds disperse by non-flying mammals in the African mainland. Sirisha et al. (2018) reported that seeds dispersal in *D. chloroxylon* is mediated by frugivorous birds and mammals. In this study, *D. chloroxylon* with globose fruit consisting of 2-8 black discoid seeds attracts local birds which feed on its pulpy part due to its palatability and in this process, they disperse seeds into different areas within the deciduous forest ecosystem.

***Hybanthus enneaspermus* (L.) F. Muell**:

It is an annual wet season herb with woody stem at base and herbaceous terminally. Flowers are solitary, axillary, pedicellate, bisexual pinkish purple and borne in leaf axils. The calyx has 5 lanceolate and ciliate sepals. The corolla has 5 pinkish unequal petals, the lower one (anterior in position) is long, broad, sub-orbicular and clawed with deep purple veins, the upper pair linear with acute apex and the lateral pair falcate with slightly recurved apex. The stamens are 5, but converge into a ring around the style; the filaments of the two anterior stamens possess thick grooved greenish nectar secreting appendages and the anthers have distinct membranous connective appendage and are longitudinally dehiscent. The pistil consists of ovoid 1-celled ovary with 11 ovules on parietal placentation and clavate style and oblique wet stigma guarded by many cellulosic fibrils which together with membrane complex act as "collecting hairs" to capture and transfer the pollen to the lateral receptive stigmatic orifice. The nectar is secreted at the flower base during anthesis; it is turbid, acidic, sugars (glucose, sucrose and fructose), and amino acids (phosphoserine, proline, glutamic acid, ethanolamine, amino n-caproic acid, valine, leucine, tryptophan and isoleucine (Bahadur et al. 1987). The fruit is a pendulous globose capsule with many ovoid seeds.

Bahadur et al. (1987) reported that *Hybanthus enneaspermus* shows early morning anthesis and close back by noon time. Jerome and Alain (2003) also reported similar schedule of flower opening and closing in *H. enneaspermus*. In this study also, similar flower opening and closing schedule is observed in *H. enneaspermus*. In this species, the flowers are open at sunrise by unfolding the broad lower petal, remain so for a few hours and close back by rolling the lower petal up thereafter. The flowers close back early by noon time if exposed to direct sunlight and by early or late afternoon if not exposed to direct sunlight. The relation of flower closing time to exposure of sunlight in this species has also been reported by Jerome and Alain (2003). In *H. enneaspermus*, the anthers dehisce during anthesis and the pollen released from the anthers is blocked by the appendages of staminal connectives, which are appressed against the style forming a cone. Nectar glands on the anterior stamens secrete nectar which is collected at the corolla base. When the flowers are in open state, only lycaenid butterflies visited the flowers for nectar regularly despite the presence of wild bees in the habitat. Among lycaenids, *Zizeeria karsandra* (Figure 1d) and *Zizula hylax* (Figure 1e) are regularly nectar foragers. The butterflies landed on the lower petal and inserted their proboscis into the corolla base to collect nectar. After nectar collection,

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they moved backwards and withdrew their proboscis to leave the flower and visit next flower either on the same or different plants. During probing, the proboscis of lycaenids contacted the stigma due to which the latter is pushed upwards slightly. If the proboscis of the probing butterfly had pollen, then the latter adheres to the wet stigma and at the same time the pollen from the already dehisced anthers gets transferred onto the proboscis and head part. In this process, either cross- or self-pollination occurs the butterfly carries the pollen, which when visits another flower for nectar collection effects pollination. The occurrence of autogamy is more likely without the requirement of any vector during flower closure. Beattie (1974) reported that *H. enneaspermus* flowers are visited by *Ceratina* bees for forage collection during which they effect pollination nototribically. Bahadur et al. (1987) reported that this plant species is foraged by beetles (*Coccinella septumpunctata*) and black ants (*Camponotus* sp.). The study indicates that *H. ennaespermus* with allogamy as the main pollination mode and autogamy as fail-safe pollination mode; the allogamy is mediated by lycaenid butterflies and hence this species is psychophilous in the ecological situation of the study site.

4. CONCLUSIONS

Diospyros chloroxylon is an evergreen dry season blooming tree species. It is dioecious with male flowers on cymose inflorescence and solitary female flowers born in the leaf axils of different individuals. In both flower sexes, rudimentary or non-functional opposite sex organ is present indicating that the plant species has evolved dioecy from hermaphroditism. Further, the male flowers offer both nectar and pollen while the female flowers offer only nectar. The honey bees were the exclusive foragers-cum-pollinators of this plant species; they visit both flower sexes effecting cross-pollination. Among honey bees, *A. cerana* and *A. florea* also resort to nectar robbery by piercing the basal part of corolla tube from outside, the behavior of which would appear to be promoting flower visitation rate to both flower sexes by them, which maximizes pollination rate when visited from the flower front side. The fruits attract local birds which while feeding on the pulpy part indulge in seed dispersal either at parental sites or non-parental sites within the deciduous forest ecosystem.

Hybanthus enneaspermus is an annual hermaphroditic herb which flourishes well during wet season. The flowers are hermaphroditic, self-compatible, allogamous and autogamous. Allogamy is functional through vector-mediated pollination while autogamy is functional through flower closure. The well-developed floral structure and mechanism controls the occurrence of autogamy and promotes the occurrence of allogamy during open state of the flowers with the involvement of lycaenid butterflies. Therefore, this plant species engages autogamy as a fail-safe mechanism during flower closure in the event of non-occurrence of pollination during open state of flowers.

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Authors contributions:

All authors contributed equally.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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